

DOCKET NO:



IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

ANNA BERGGREN, ET AL.

: EXAMINER: PRATT

SERIAL NO: 09/926,586 :

FILED: NOVEMBER 21, 2001

: GROUP ART UNIT: 1761

FOR: NEW COMPOSITION :

DECLARATION UNDER 37 C.F.R. §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Anna Berggren state that:

1. I am a named coinventor of the above-identified application.

2. That I have been employed by Probi AB for 9 years as a
res. Scientist in the field of nutrition.

3. The following stability study was performed and shows that not all species within the Lactobacillus genera have the desired stability. Indeed, the study discussed below shows that specific strains of *L. plantarum* 299v, *L. paracasei* 9700:2, and *L. rhamnosus* 271 are able to survive in the sports drink with the micronutrients during the 5 week study.

4. **Stability tests (during 5 weeks) of four different bacteria present in sports drink**

5. **Object:**

6. Stability test at storage (7°C) during 5 weeks of an oat base (7%) fermented with four different bacteria as presented below in micronutrient composition. The micronutrients

present in the sports drink correspond to the micronutrients as disclosed in the international application WO 00/70972 on p. 11 lines 4-14 during 5 weeks.

7. Methodology and contents:

8. An oat base (7% by weight) was fermented with the respective bacteria (*Lactobacillus plantarum* 299v, *Lactobacillus paracasei* 8700:2, *Lactobacillus rhamnosus* 271, *Lactobacillus reuteri protectis*) at 37°C over night until a pH<4 was achieved. The fermented oat base was kept in refrigerator until usage.

9. A sports drink was prepared containing the following micronutrients per 100 g :

Ascorbic acid	80 mg
Vitamin E,	40 mg
β-carotene,	2 mg
Vitamin B6 (pyridoxine),	2 mg
copper,	0.1 mg
magnesium,	15 mg
manganese,	0.2 mg
selenium	10 µg
Zinc	1 mg

10. In addition to the micronutrients above the sports drink contained per 100 g drink:

Protein	2g
Carbohydrate	13 g
Fat	<0.1 g
Sodium	0.01 g

11. The carbohydrates were sucrose (4g/100g), glucose (6g/100g) and fructose (2g/100g). A fruit preparation of blueberry, raspberry and lemon was added for a pleasant taste.

12. After the above components had been mixed, the same was heated to a temperature high enough to eliminate any bacteria or microorganisms present in the drink. The fermented oat base was then added until the total bacterial content was the following in each drink:

13. Total bacterial content in prepared sports drink;

1. *Lactobacillus plantarum* 299v, about 5×10^7 cfu/ml.
2. *Lactobacillus paracasei* 8700:2, about 5×10^7 cfu/ml.
3. *Lactobacillus rhamnosus* 271, about 5×10^7 cfu/ml.
4. *Lactobacillus reuteri protectis*, about 5×10^5 cfu/ml.

14. Each sports drink was allowed to stand in a refrigerator at 7°C for 5 weeks. The bacterial content was checked in view of stability weekly from the start. A hygiene analysis was also performed weekly in order to check presence of other growing bacterial species.

1) Results of analysis; *Lactobacillus plantarum* 299v

Analysis date	Storage time	No. cfu/ml	Hygiene analysis
21-02-2007	0 sample	$5,5 \times 10^7$	-
28-02-2007	1 w	$5,3 \times 10^7$	OK
07-03-2007	2 w	$8,7 \times 10^7$	OK
14-03-2007	3 w	$6,7 \times 10^7$	OK
21-03-2007	4 w	$9,8 \times 10^7$	OK
28-03-2007	5 w	$6,2 \times 10^7$	OK

2) Results of analysis; *Lactobacillus paracasei* 8700:2

Analysis date	Storage time	No. cfu/ml	Hygiene analysis
21-02-2007	0 sample	$9,7 \times 10^7$	-
28-02-2007	1 w	$8,5 \times 10^7$	OK
07-03-2007	2 w	$2,2 \times 10^8$	OK
14-03-2007	3 w	$9,8 \times 10^7$	OK
21-03-2007	4 w	$1,9 \times 10^8$	OK
28-03-2007	5 w	$1,5 \times 10^8$	OK

3) Results of analysis; *Lactobacillus rhamnosus* 271

Analysis date	Storage time	No. cfu/ml	Hygiene analysis
21-02-2007	0 sample	$7,7 \times 10^7$	
28-02-2007	1 w	$6,5 \times 10^7$	OK
07-03-2007	2 w	$7,8 \times 10^7$	OK
14-03-2007	3 w	$9,7 \times 10^7$	OK
21-03-2007	4 w	$1,1 \times 10^8$	OK
28-03-2007	5 w	$1,3 \times 10^8$	OK

4) Results of analysis; *L. reuteri protectis*


Analysis date	Storage time	No. cfu/ml	Hygiene analysis
21-02-2007	0 sample	$3,1 \times 10^4$	
28-02-2007	1 w	about 2×10^3	OK
07-03-2007	2 w	$1,3 \times 10^3$	OK
14-03-2007	3 w	$5,9 \times 10^2$	OK
21-03-2007	4 w	$<10^5$	OK
28-03-2007	5 w	$2,3 \times 10^2$	OK

15. **Conclusion:** From the above it can be seen that the bacteria *Lactobacillus plantarum* 299v, *Lactobacillus paracasei* 8700:2, *Lactobacillus rhamnosus* 271 remain stable during the entire stability study of 5 weeks, i.e. the bacterial content is not decreased. On the other hand, *Lactobacillus reuteri protectis* does not remain stable. Its CFU is decreased considerably during the five-week storage, which means that it is not suitable for a sports drink containing the specific micronutrients. The above stability study shows that the specific environment of 17 micronutrients is not suitable for all species of *Lactobacillus*. In the case it is not suitable for *Lactobacillus reuteri*.

16. The undersigned declare further that all statements made herein are of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signature

Date


 19 July 2007